GLUCOSE-DEPENDENT INSULIN INHIBITION OF KETONE BODY FORMATION FROM LONG-CHAIN FATTY ACIDS IN THE PERFUSED LIVERS OF FASTED RATS

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ABSTRACT: The data presented in this report show a direct effect of insulin on impairment of ketone body production in perfused livers from fasted rats. The data also show that physiological levels of insulin alone or glucose alone are not sufficient to cause an impairment in ketogenesis. Only when insulin and glucose are both present at levels seen in infected rats is ketone body production impaired.

Stimulus for this study arose from the fact that the plasma of infected-fasted rats has low ketone body concentration and high insulin levels (1). Among the infections which have been studied in the rat are streptococcus pneumoniae, Francisella tularensis, salmonella typhimurium. Foster (2) demonstrated that the injection of insulin into fasted rats caused a rapid transient decline in ketone body concentration. Haft and Miller (3) showed a similar inhibition of ketone body production by insulin in perfused livers from diabetic rats. Poledne and Mayes (4) mention in an abstract with no experimental details that the addition of insulin to perfused livers from starved normal rats resulted in a reduction in ketone body formation. Other authors, however, have found that insulin does not reduce ketone body formation in the perfused rat liver (5, 6). This paper presents direct evidence for the combined role of glucose and insulin in causing a reduction of ketone body formation from oleic acid in perfused livers from normal fasted rats.

METHODS

Liver perfusions were performed as described by Wannemacher et al. (5) with the modifications described below. The perfusion medium consisted of 70 ml Krebs-Ringer bicarbonate buffer (pH 7.4), 30 ml of washed sheep erythrocytes, 3 g of fatty acid-poor bovine serum albumin, 500 U of heparin and in those perfusions where insulin was present, enough insulin was added to give a concentration of 25 μunits/ml. In experiments where no glucose was added the basal glucose level was approximately 50 mg/100 ml. In those experiments where glucose was added, the concentration of glucose averaged 150-200 mg/100 ml. The atmosphere in the closed perfusion system consisted of 95% O_2 and 5% CO_2. Perfusion of the liver was maintained at about 1 ml/min per g; the perfusate was recycled. At the end of a 45-min equilibration period, 2.5 ml of a 20 mM solution of oleic acid and insulin, as described above was added. A 3-ml sample of the perfusate was removed at 0, 15, 30, 45, and 60 min or at 0, 10, 20, 30, 40, 50, and 60 min, and analyzed for ketone bodies. The rate of ketogenesis was calculated from the slope of the plot of total ketones in the media vs. time.

Ketone bodies were determined as described by Neufeld et al. (6) and glucose as described by Sudduth et al. (7).

RESULTS

If glucose, without exogenous insulin is added to the perfusate so that its concentration is at or close to that seen in the blood of normal fasted rats, 150-200 mg/100 ml, there is no effect on hepatic ketogenesis (Fig. 1). Additionally, if insulin, without added glucose, at a level close to or equal to 25 μU/ml is added to the perfusate, there is no inhibition of ketone body formation. If, however, the liver is perfused with a medium which contains a high glucose concentration (150-200 mg/100 ml) and insulin at a level of 25 μU/ml, there is a 40% inhibition in the ability of the liver to form ketones.

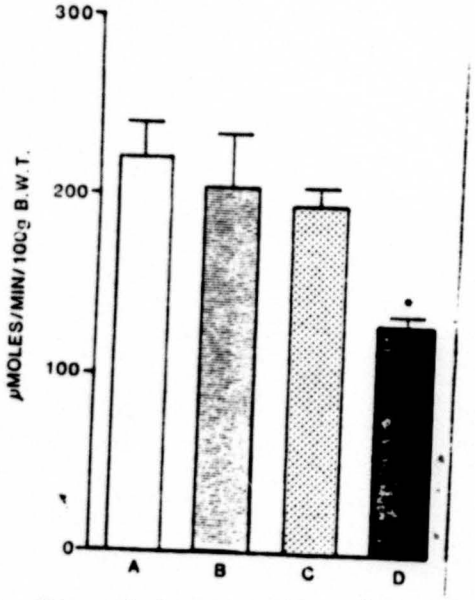

Figure 1. Effect of glucose, insulin, and glucose + insulin on ketone body formation from oleic acid in the perfused livers from fasted rats. *$P \leq 0.05$ vs. A, B, and C. Oleic acid □ ; Oleic acid + glucose (200 mg/100 ml) ▤ ; Oleic acid + insulin (25 μU/ml) ▦ ; Oleic acid + insulin (25 μU/ml), and glucose (200 mg/100 ml) ■ .

DISCUSSION

Data presented by Neufeld et al. (1) and Kaminski et al. (8) indicated an apparent important role

1) In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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for insulin in the reduction of ketone body formation in the livers of rats which were infected or in which an inflamed state was induced. Other indirect evidence for the role of insulin in states of low ketone body concentration has been presented by Woodside and Heimberg (9) who observed that the addition of anti-insulin serum increased the rate of ketogenesis. They also observed that the addition of glucose to the perfusate depressed ketogenesis, a situation not found to be so in the work reported here. Their work, however, was primarily with the livers from diabetic rats. Poledne and Mayes (4) reported in an abstract that insulin decreased ketone body production.

Miller (10) demonstrated a direct effect of insulin on hepatic gluconeogenesis with no mention of ketogenesis. Other investigators, Penhos et al. (11) and Haft (12) were unable to show any effect of insulin on ketogenesis. Haft concluded that the decrease in ketone body concentration observed following the in vivo administration of insulin could not be explained by a direct inhibition of hepatic fatty acid oxidation.

The discrepancy between Haft's results and others' with the data presented in this report can, perhaps, be explained by the difference in the concentration of glucose in the perfusate. In our hands, when no glucose is added to the perfusate, the endogenous concentration of glucose in the perfusate is slightly in excess of 50 mg/100 ml. Added insulin with this concentration of glucose caused no inhibition of ketone body formation from oleic acid. If, however, sufficient glucose is added so that its concentration in the perfusate is close to that observed in the fasted state of the rat, inhibition of ketogenesis from oleic acid by added insulin is observed.

The data presented in this report confirm the direct effect of insulin on ketogenesis and add importance to the observed increase in plasma insulin in fasted-infected rats (2). In conclusion, this study demonstrates the glucose-dependent role of insulin in the suppression of hepatic ketosis.

REFERENCES

1. Neufeld HA, Pace JG, Kaminski MV, George DT, Jahrling PB, Wannemacher RW Jr, Beisel WR 1980 A probable endocrine basis for the depression of ketone bodies during infectious or inflammatory state in rats. Endocrinology 107:596
2. Foster DW 1967 Studies on the ketosis of fasting. J Clin Invest 46:1283
3. Haft DE, Miller LL 1958 Alloxan diabetes and demonstrated direct action of insulin on metabolism of isolated perfused rat liver. Am J Physiol 192:33
4. Poledne R, Mayes PA 1970 Lipolysis and the regulation of fatty acid metabolism in the liver. Biochem J 119:47P.
5. Wannemacher RW Jr, Pace JG, Beall FA, Dinterman RE, Petrella VJ, Neufeld HA 1979 Role of the liver in regulation of ketone body production during sepsis. J Clin Invest 64:1565
6. Neufeld HA, Pace JA, White FE 1976 The effect of bacterial infections on ketone concentration in rat liver and blood and on free fatty acid concentrations in rat blood. Metabolism 28:877
7. Sudduth NB, Widish JR, Moore JL 1970 Automation of glucose measurement using ortho-toluidine reagent: comparison of values determined by automated ferricyanide and ortho-toluidine 53:181
8. Kaminski, MV Jr, Neufeld HA, Pace JG 1979 Effect of inflammation and noninflammatory stress on plasma ketone bodies and free fatty acids and on glucagon and insulin in peripheral and portal blood. Inflammation 3:289
9. Woodside WF, Heimberg M 1976 Effects of anti-insulin serum, insulin, and glucose on output of triglycerides and on ketogenesis by the perfused rat liver. J Biol Chem 251:13
10. Miller LL 1965 Direct actions of insulin, glucagon, and epinephrine on the isolated perfused rat liver. Fed Proc 24:737
11. Penhos JC, Wu CH, Lemberg A, Daunas J, Brodoff B, Sodero A, Levine R 1968 The effect of insulin on the metabolism of lipids and on urea formation by the perfused rat liver. Metabolism 17:246
12. Haft DE 1968 Metabolism of a tracer dose of palmitate-1-C^{14} by liver of normal and diabetic rats perfused with and without addition of insulin. Metabolism 17:818

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A114703	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Glucose-dependent insulin inhibition of ketone body formation from long-chain fatty acids in the perfused livers of fasted rats		5. TYPE OF REPORT & PERIOD COVERED Interim
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Harold A. Neufeld, Judith G. Pace, Francis Beall, and David L. Bunner		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases, SGRD-UIS Fort Detrick, Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS S10-AQ-197
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Office of the Surgeon General Department of the Army, Washington, DC 20314		12. REPORT DATE 13 April 1982
		13. NUMBER OF PAGES 2
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution unlimited - Approved for public release
(Rapid paper)

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt.
To be published in Journal of Endocrinology.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Insulin, glucose, ketone bodies, liver perfusion

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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